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# CHRONIC EXPOSURE STUDIES WITH MONOMETHYL-HYDRAZINE

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13. ABSTRACT

This report was presented at the Proceedings of the 2nd Annual Conference on Environmental Toxicology, sponsored by the SysteMed Corporation and held in Fairborn, Ohio on 31 August, 1 and 2 September 1971. Major technical areas discussed included toxicological evaluation of volatile halogenated compounds, protection of the public against air pollution and toxicological problems with aircraft, missiles, and space vehicles.

Key words:

Continuous exposure
Pathology
Toxicological screening
Gas chromatography
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# CHRONIC EXPOSURE STUDIES WITH MONOMETHYLHYDRAZINE

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The manufacture and use of monomethylhydrazine (MMH) as a rocket fuel has increased over the past 10 years. The acute health hazards from handling this highly reactive compound were well defined, but although its usage was increasing little was known about its chronic exposure effects. The current industrial threshold limit value (TLV) of 0. 2 ppm was established by analogy with hydrazine and unsymmetrical dimethylhydrazine. Jacobson et al. (1955) had shown that the acute CNS effects from MMH exposure were intermediate to its two analogs. Additional acute exposure studies (Haun et al., 1971) revealed that the CNS effects and resulting death were dose-related with MMH following Haber's Law of CT = K.

A series of 6-month MMH chronic exposures to four animal species was undertaken to evaluate the safety factor and appropriateness of the current TLV for health of workmen. Exposures were conducted on a 6-hour/day 5-day/week basis at air concentrations of 0. 2, 1, 2, and 5 ppm MMH in four experiments. Another experiment was conducted which provided a continuous exposure of 0. 2 ppm to animals for consideration of exposure limits for use in missile silos, spacecraft, or other confined spaces. The weekly dose of MMH in ppm-hours for each experiment is shown in table I. The 2 and 5 ppm MMH exposure experiments were made first and the results were reported by Haun (1970) at this conference last year.

Each of the experimental animal groups, as well as their controls, consisted of 8 beagle dogs, 4 rhesus monkeys, 50 Wistar strain rats, and 40 ICR mice. All animals were female except for male rats. The exposures were conducted in the Thomas Domes which were operated at 725 mm Hg pressure to avoid leakage of MMH into the laboratory environment. The exposure chamber effluent air was passed through a high volume water cooled vacuum pump which prevented discharge of the residual MMH to outdoor air by reaction with the water. The chamber MMH concentrations were continuously monitored and controlled using a colorimetric method with an AutoAnalyzer (Geiger, 1967).

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The experimental animals were weighed biweekly during the studies and a series of 15 clinical chemistry and eight hematology tests was conducted on the same schedule. On conclusion of the experiments, the animals were killed for gross and histopathologic examination. Bone marrow studies on dogs were also performed at this time. At the end of the first series of experiments, half of the exposed and control dogs were held for 30 days postexposure observation to determine reversibility of the noted effects. The second series of experiments was extended four weeks to permit additional blood sampling because two sampling periods near the end of the experiment were unavoidably omitted.

TABLE I

MMH WEEKLY DOSE EQUIVALENTS

| Chamber Concentration (ppm) | Type of Exposure | Dose ppm-Hours |
|---|---|---|
| 0.2 | Intermittent* | 6 |
| 0.2 | Continuous | 33.6 |
| 1.0 | Intermittent* | 30 |
| 2.0 | Intermittent* | 60 |
| 5.0 | Intermittent* | 150 |

*6 hours/day - 5 days/week

## EXPERIMENTAL RESULTS

Consistent dose-related effects were seen at all exposure levels, including the 0.2 ppm MMH continuous exposure. Deaths occurred only in mice at the two highest MMH concentrations. The mortality percentages were 27% at 5 ppm and 15% for the 2 ppm MMH exposure group. Mortality in mice at lower MMH exposure concentrations was comparable to that of the control groups.

Rat growth was significantly depressed in the 2 and 5 ppm MMH exposures as shown in figure 1. Body weight data for the lower MMH exposure concentrations also showed evidence of dose-dependent effects at 1 ppm intermittent exposure and at 0.2 ppm MMH continuous exposure. At no time interval were the mean weights of the rats exposed at the lowest dose level (0.2 ppm intermittent) statistically different from those of the control group. Differences, significant at the 0.01 level, were seen from the first to ninth week for the 1.0 ppm MMH intermittently exposed group and at weeks seven, nine, and 13 in the case of the 0.2 ppm continuous exposure rats, as seen in figure 2. High room temperatures caused by heating

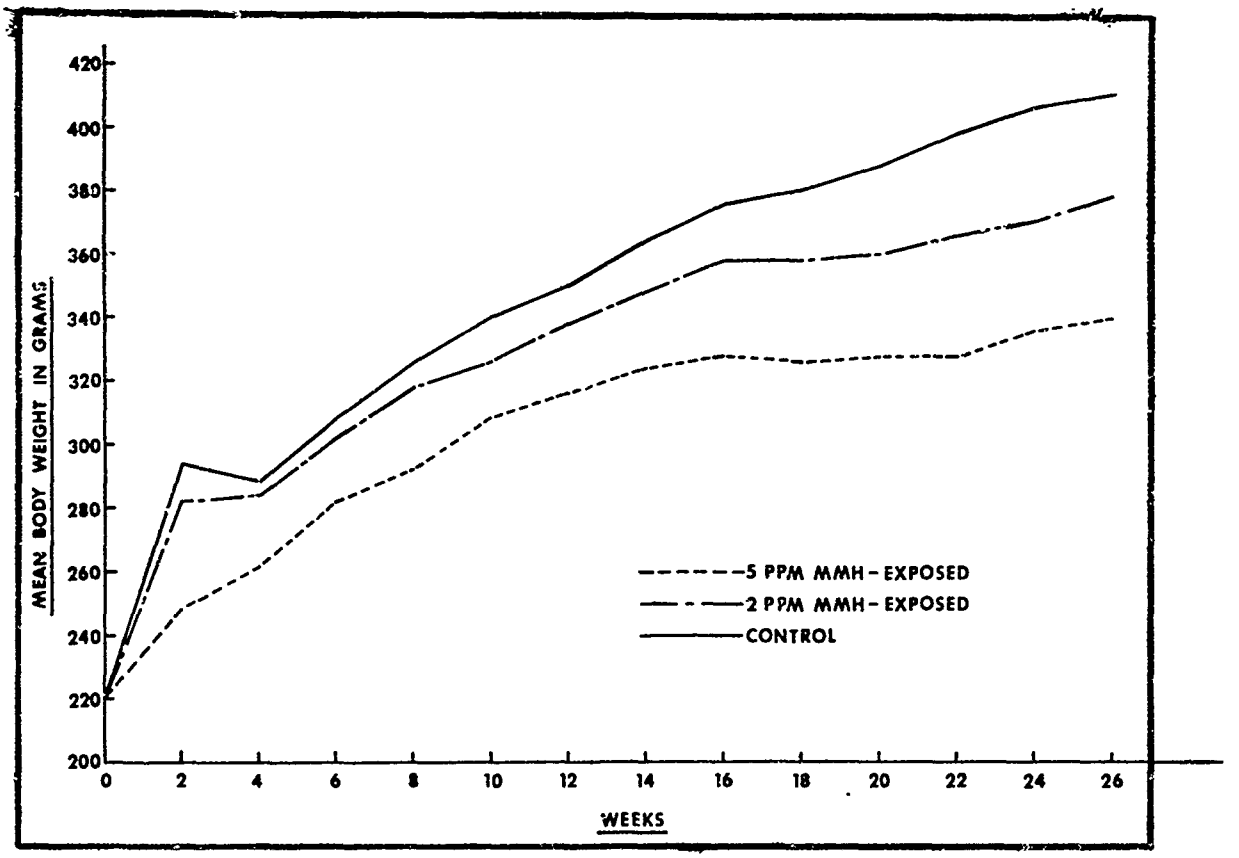


Figure 1. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON RAT GROWTH - GROUP I.

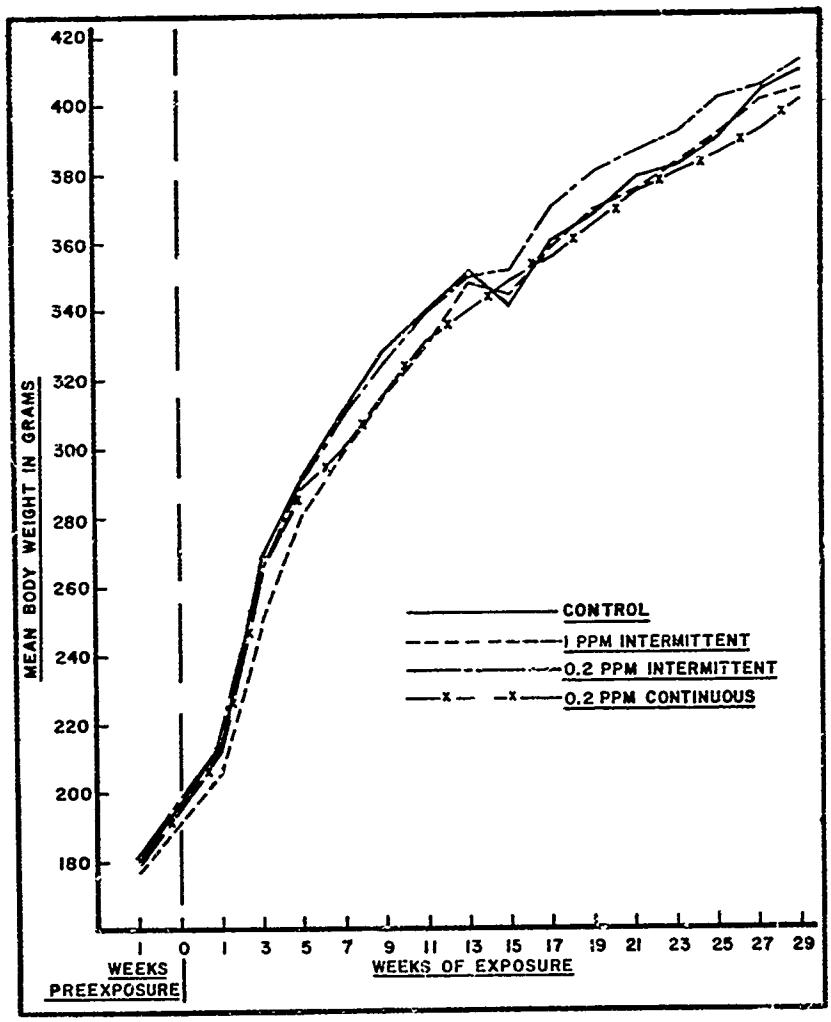


Figure 2. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON RAT GROWTH - GROUP II.

equipment malfunction occurred in the laboratory area containing the control animal chamber. Unfortunately, the control rats experienced actual weight loss resulting from the heat stress and comparisons between the low level MMH exposed rats and controls are valueless after the thirteenth week.

The majority of induced chronic effects on the animals were related to reaction of MMH with circulatory red blood cells. The effects were greatest in dogs but also occurred in monkeys. Hematology studies were not conducted on the rodents. Monomethylhydrazine produced a dose-related increase in methemoglobin, as shown in figure 3, for the two highest exposure levels. The methemoglobin increase, although not plotted, was also statistically significant at lower doses.

Figure 4 shows the effect of MMH exposure on dog red blood cell counts at 2 and 5 ppm, and figure 5 shows this effect at lower doses. The top unbroken line represents the mean red blood cell count of the group of eight control dogs in each figure during the six-month exposure period. The dose-response relation is clearly seen in figure 6 where the mean red blood cell values for each of the five exposure groups are shown at the six-month sampling point. The weekly MMH dose given to the animals in the 1 ppm intermittent exposure group is 30 ppm-hours and for the 0.2 ppm continuous exposure group the dose is 33.6 ppm-hours. Thus, the animals in these two exposure groups received essentially the same weekly dose of MMH and are very comparable in their response to exposure as shown in this figure. Therefore, in this and all other dose comparison curves, the 0.2 ppm continuous exposure results are plotted at the 1 ppm point. The effect of MMH on monkey red blood cell counts is shown in figure 7 for the first series of experiments.

Biweekly mean values for dog hematocrits are shown in figure 8 for the second series of six-month exposures. Again the solid line represents the control group, showing a small but significant effect at 0.2 ppm intermittent MMH exposure and a comparable effect at 0.2 ppm continuous and 1 ppm intermittent exposure. The dose response for hematocrit values of dogs is shown in figure 9. This relationship follows the plotted curve even better than the red blood cell counts. Mean biweekly hemoglobin values and the dose response are shown in figures 10 and 11, respectively. Again the various exposure groups exhibit a clear dose effect relationship with no apparent threshold effect level.

The effect of MMH exposure on red blood cell fragility is seen in figures 12 and 13, which are composite fragilograms for the exposed and control dogs. Values plotted for each curve on these graphs represent the mean values of five monthly determinations for each group. There was very little variation among animals in each group during the entire experimental period. There is a very definite shift toward increased initial hemolysis with increasing MMH dosage as shown in figure 14 which presents the percent hemolysis of RBC's in a 0.60% salt solution.

Blood samples taken from all dogs and monkeys at three, four, five, and seven months were examined microscopically for the presence of Heinz bodies. Group mean values from all sampling periods were always relatively low, but positive for

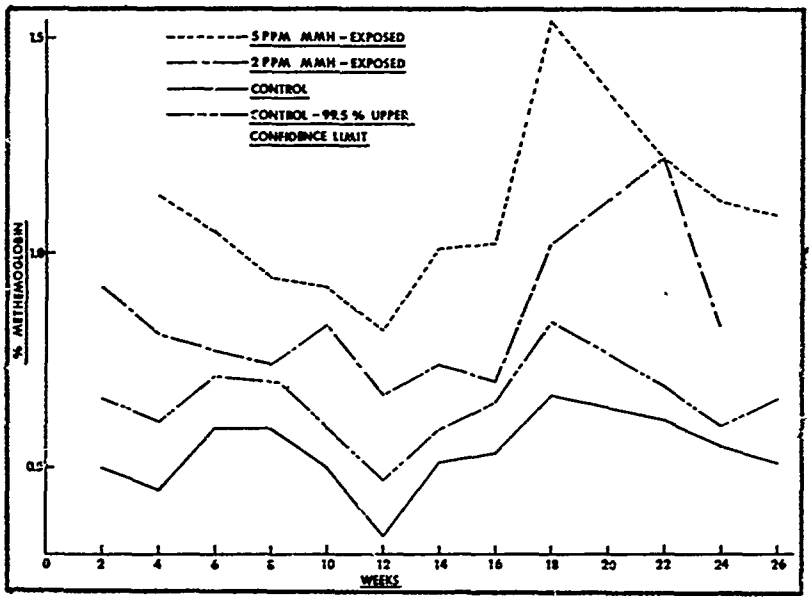


Figure 3. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON METHEMOGLOBIN FORMATION IN DOGS.

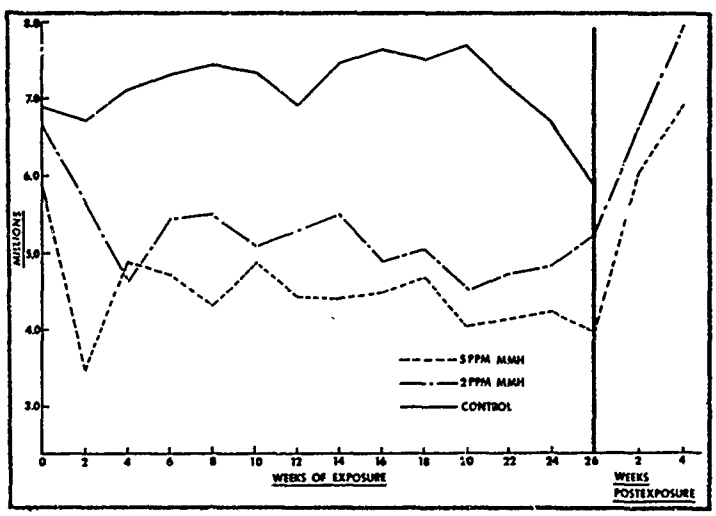


Figure 4. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON DOG RED BLOOD CELL COUNTS - GROUP I.

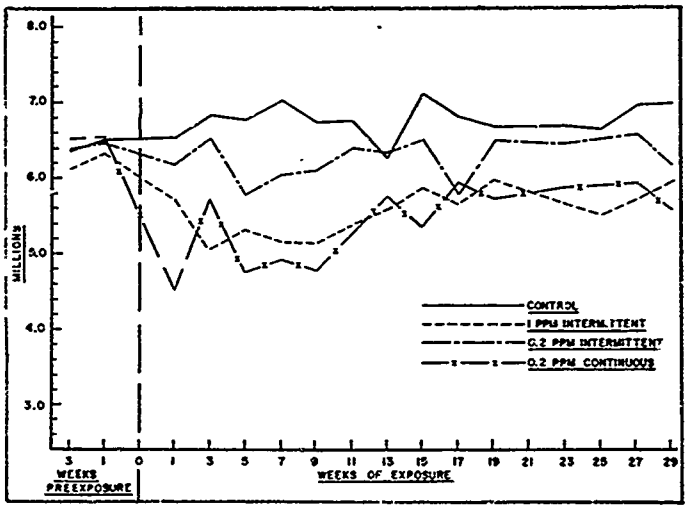


Figure 5. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON DOG RED BLOOD CELL COUNTS - GROUP II.

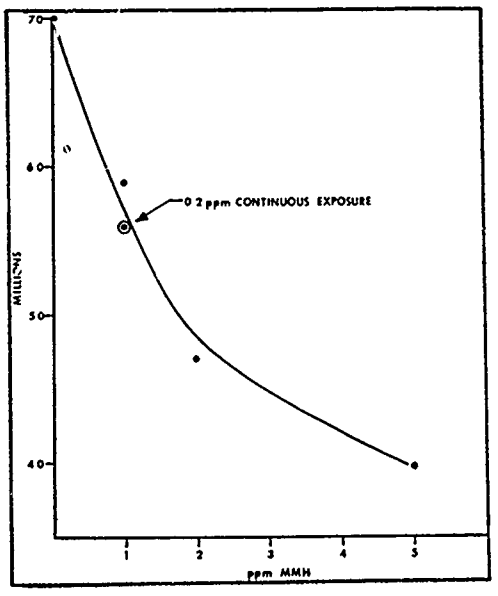


Figure 6. MEAN RED BLOOD CELL COUNTS IN DOGS EXPOSED TO VARIOUS CONCENTRATIONS OF MONOMETHYLHYDRAZINE FOR SIX MONTHS.

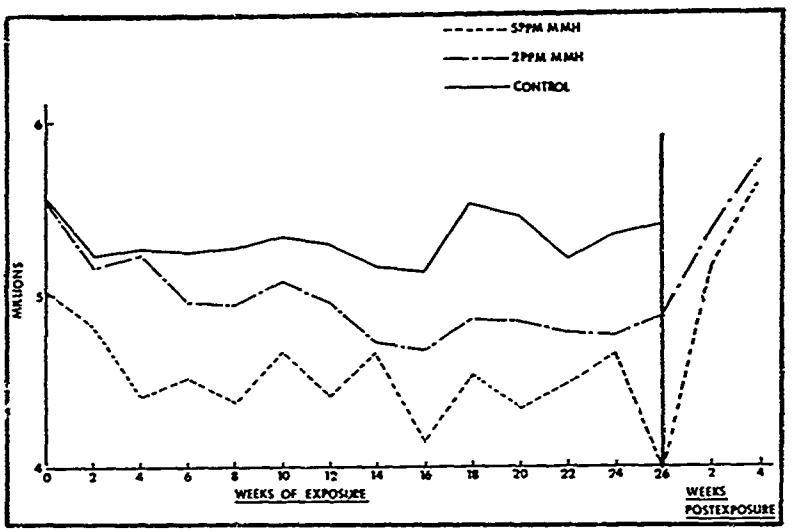


Figure 7. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON MONKEY RED BLOOD CELL COUNTS - GROUP I.

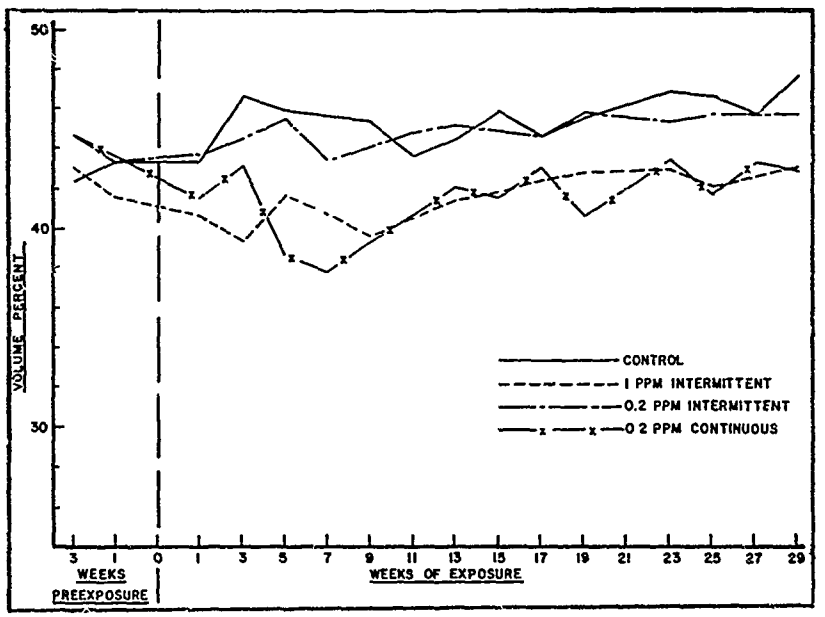


Figure 8. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON DOG HEMATOCRIT LEVELS - GROUP I.

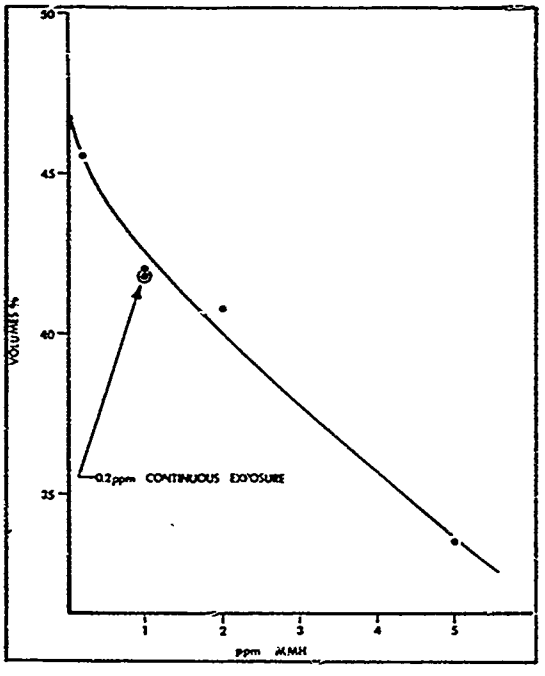


Figure 9. MEAN HEMATOCRIT VALUES IN DOGS EXPOSED TO VARIOUS CONCENTRATIONS OF MONOMETHYLHYDRAZINE FOR SIX MONTHS.

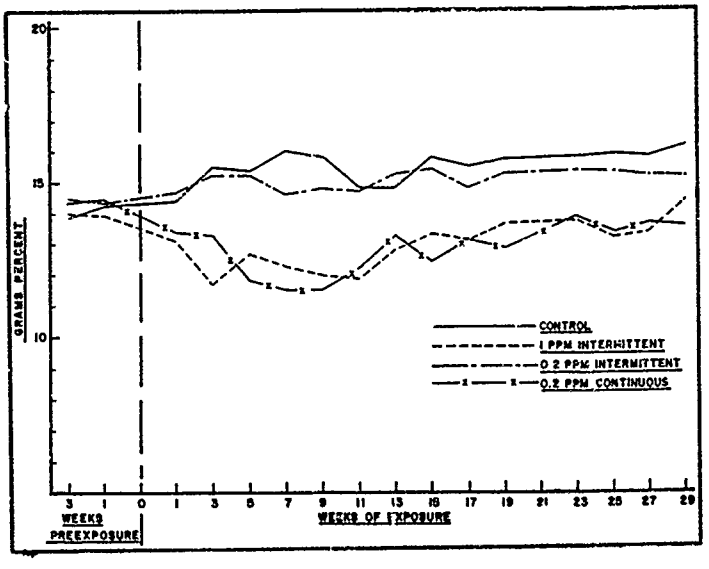


Figure 10. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON DOG HEMOGLOBIN VALUES - GROUP II.

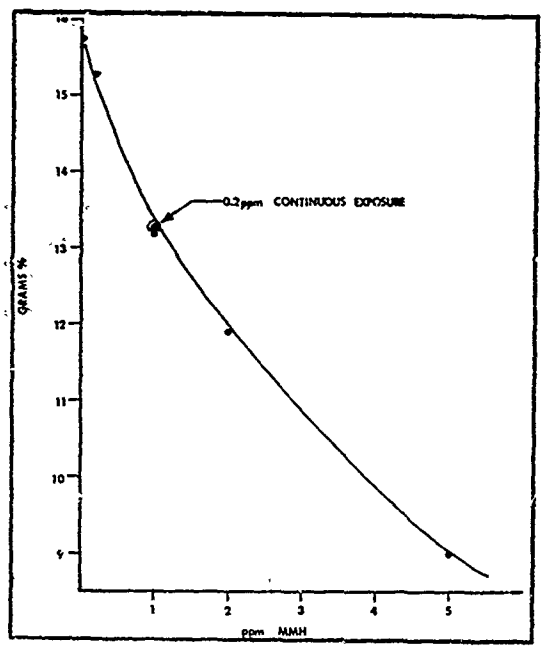


Figure 11. MEAN HEMOGLOBIN VALUES IN DOGS EXPOSED TO VARIOUS CONCENTRATIONS OF MONOMETHYLHYDRAZINE FOR SIX MONTHS.

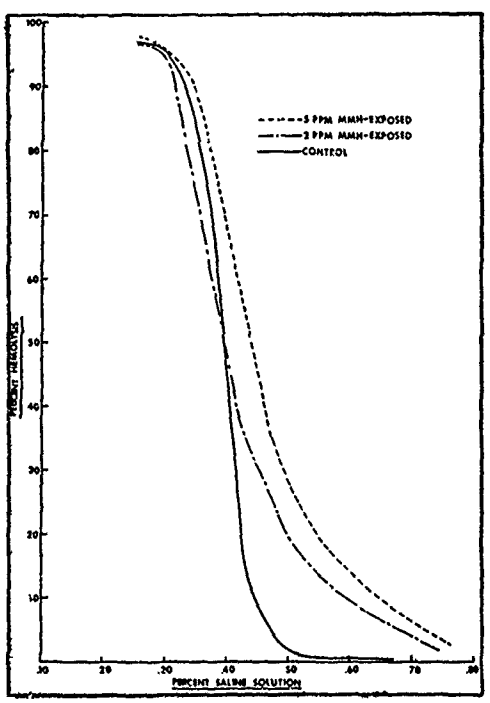


Figure 12. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON RED BLOOD CELL FRAGILITY IN GROUP I DOGS.

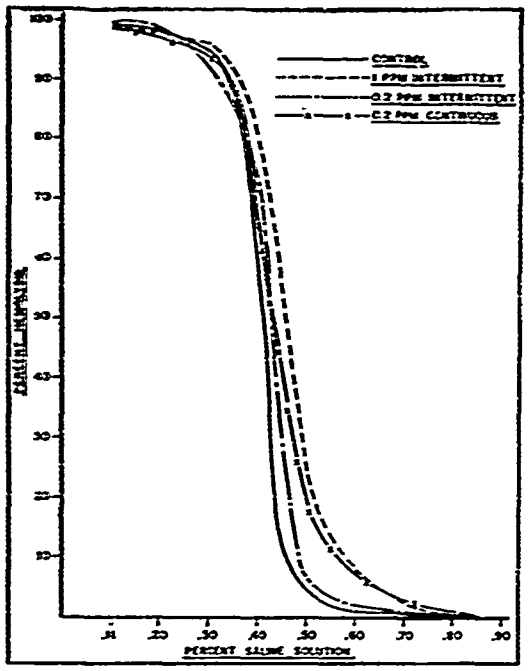


Figure 13. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON RED BLOOD CELL FRAGILITY IN GROUP II DOGS.

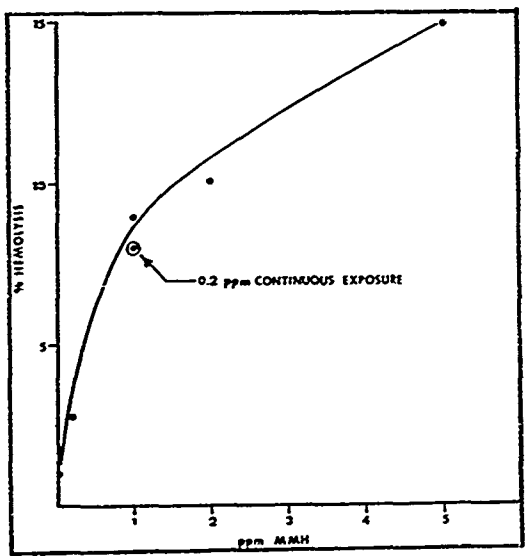


Figure 14. EFFECT OF MONOMETHYLHYDRAZINE EXPOSURE ON THE FRAGILITY OF DOG RED BLOOD CELLS IN A 0.6% SALT SOLUTION.

all exposed groups. Mean values of from one to five Heinz bodies in 100 red blood cells were found in each sample from MMH exposed animals. No dose- or species-related effects were evident. Overall assessment of the results of this test suggests, however, that minimal hemolytic effects were induced in monkeys as well as dogs as a result of low level exposures to MMH.

The dose effect of MMH on hematologic values in monkeys is shown in figure 15. Although the MMH induced effects are not as great as those seen in dogs, they are significant in terms of stress on the hemopoietic system.

Examination of clinical chemistry data, consisting of 15 separate determinations collected on a regular biweekly schedule for dogs and monkeys during the course of the study, revealed that mean bilirubin, alkaline phosphatase, and total inorganic phosphorus values for all exposed dog groups were, for the most part, statistically higher than control values.

Serum bilirubin and alkaline phosphatase levels were significantly elevated in all dog exposure groups at all sampling periods from three weeks to the conclusion of the study. Figures 16 and 17 present group mean values for each of these determinations. Dose-dependent effects are noticeable in both figures. Values for dogs exposed to the two highest MMH concentration levels were consistently higher than those recorded for the lowest MMH concentration exposure group. To a lesser extent, the latter values were repeatedly higher than control. Total inorganic phosphorus results, figure 18, were less pronounced, particularly for dogs exposed to the lowest concentration level, but are indicative, as are the abnormally high bilirubin and alkaline phosphatase levels, of the intrahepatic choleostasis produced in dog livers as a result of chronic exposure to MMH.

All exposed and control animals were sacrificed at the conclusion of the study and submitted for gross necropsy. Major organs from all dogs, monkeys, 10 rats, and 10 mice from each group were saved for histopathologic examination. The results of these examinations are reported by Dr. Kroe.

Bone marrow samples taken from MMH exposed dogs in each experiment were examined for their myeloid and erythroid elements. Figure 19 shows the dose-related decrease in M/E ratio with increasing erythropoietic activity. Although there was greater variation in the response of the dogs continuously exposed to MMH at 0.2 ppm, the mean M/E ratio is almost identical with that of the dogs given a comparable dose of 1 ppm MMH on an intermittent schedule.

It may well be asked where man fits in the spectrum of species responses seen with MMH chronic exposure. In a study of the in vitro formation of methemoglobin by MMH (Leahy, 1970), blood samples from four species were compared to determine their equilibrium conversion rates for oxyhemoglobin. In this study, man was found to rank next to the dog in susceptibility with a higher conversion equilibrium than the rat and monkey.

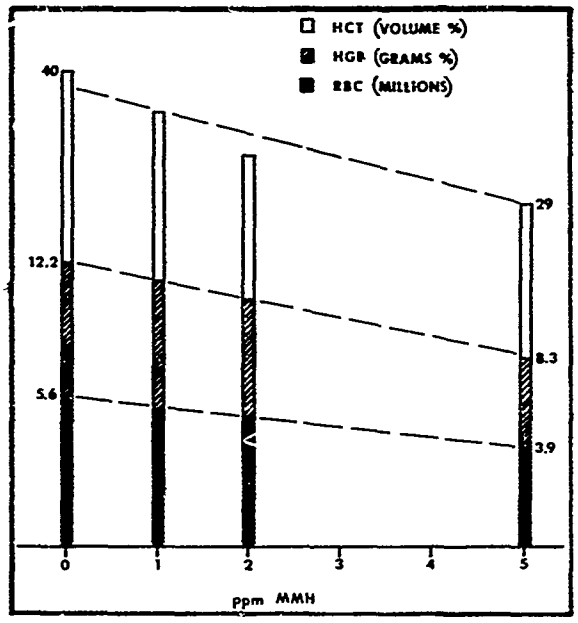


Figure 15. EFFECT OF SIX MONTH EXPOSURE TO MONOMETHYLHYDRAZINE ON HEMATOLOGIC VALUES IN MONKEYS.

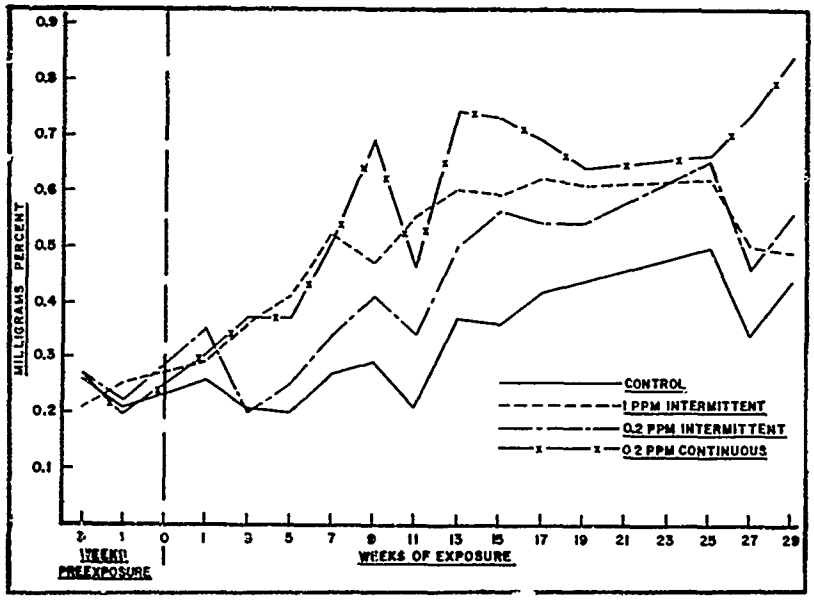


Figure 16. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON SERUM BILIRUBIN LEVELS IN DOGS.

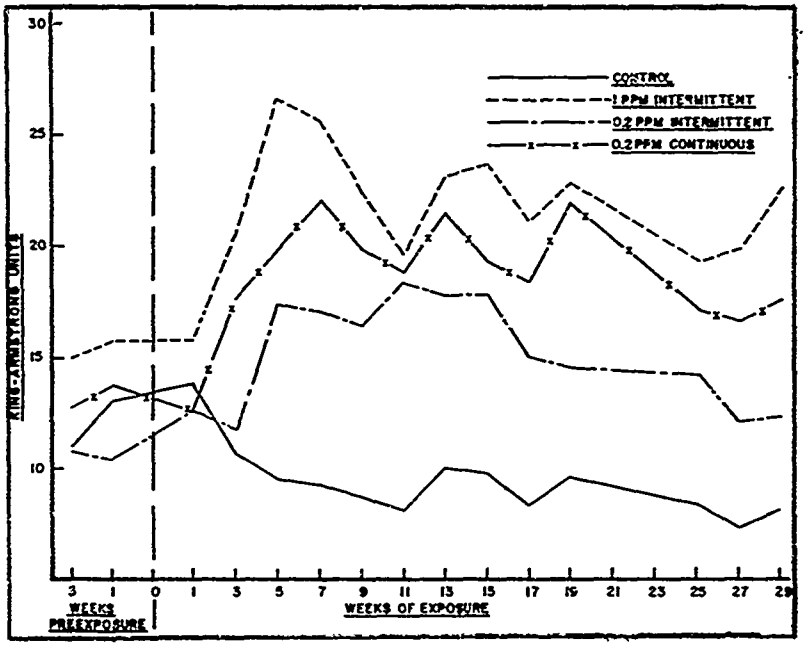


Figure 17. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON SERUM ALKALINE PHOSPHATASE LEVELS IN DOGS.

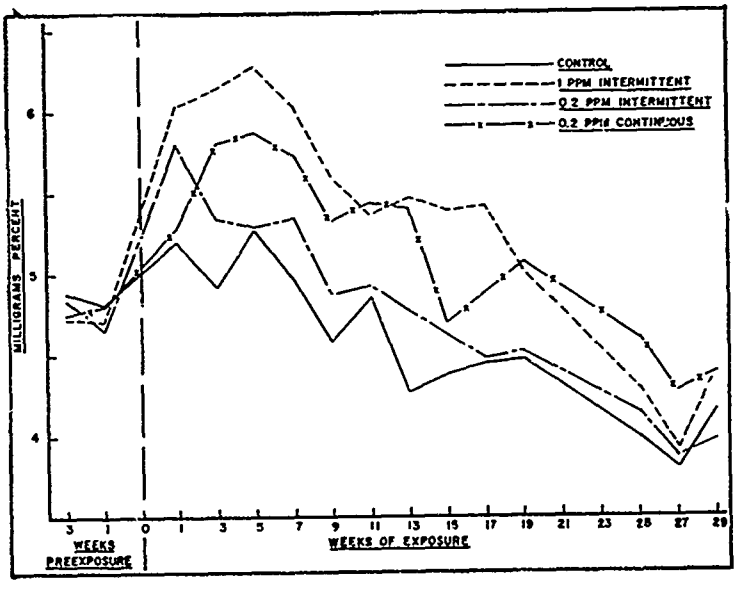


Figure 18. EFFECT OF CHRONIC MONOMETHYLHYDRAZINE EXPOSURE ON SERUM PHOSPHORUS LEVELS IN DOGS.

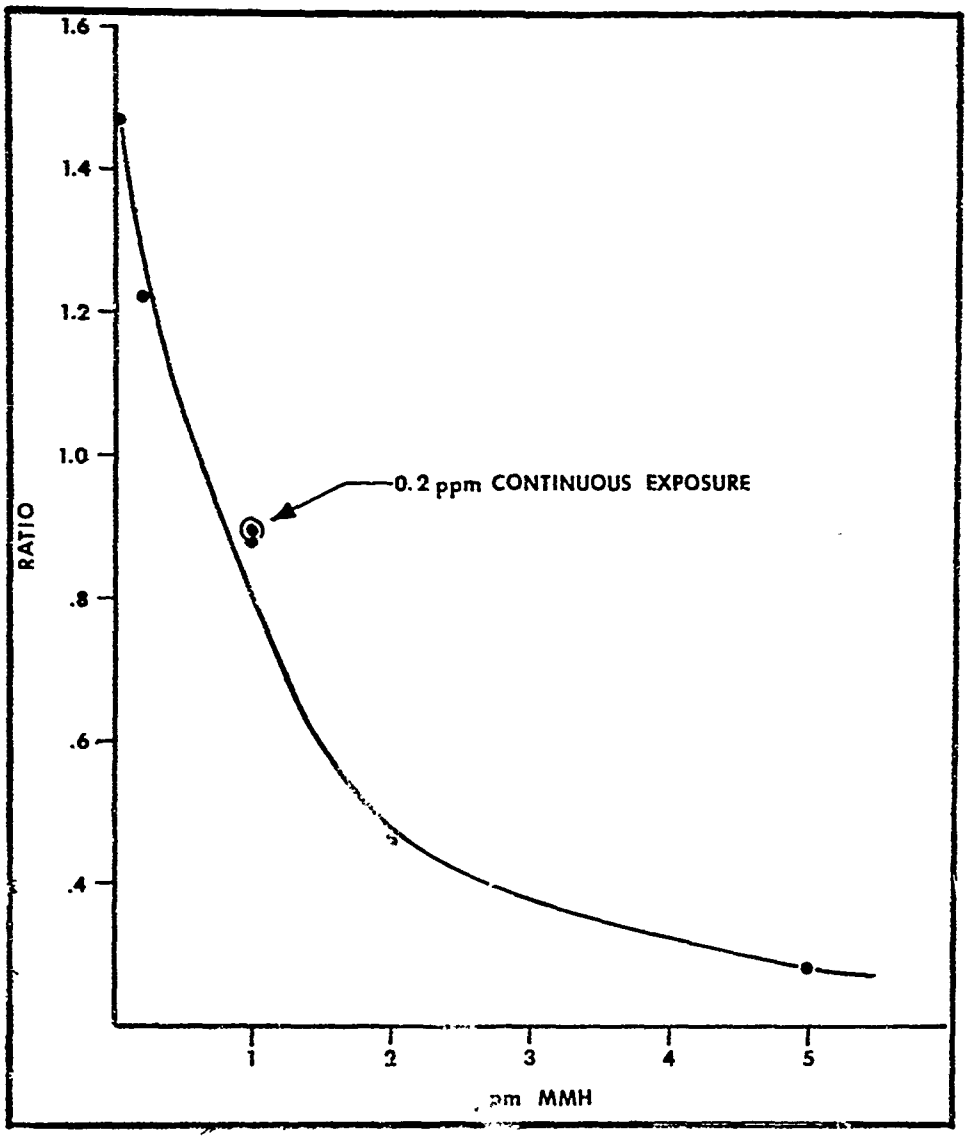


Figure 19. MEAN MYELOID/ERYTHROID RATIOS IN BONE MARROW OF DOGS AFTER SIX MONTHS EXPOSURE TO MONO-METHYLHYDRAZINE.

The results of these experiments have shown that MMH produces a dose-related hemolytic anemia with Heinz body formation for which there appears to be no threshold effect level. The anemia is reversible with removal from further exposure at least up to a level of 5 ppm intermittent exposure. These studies were performed on a 30-hour week basis but can be factored for interpretation of 40-hour weekly exposures because of the established dose-effect relationship. For use in establishing continuous exposure limits for confined spaces such as missile silos, consideration should be given to variations in concentration which could considerably shift the exposed people down the effect curve. Consideration should also be given to the effect of MMH on people with preexisting blood dyscrasias or hemolytic traits. In view of this risk, we believe the industrial TLV should be reexamined and consideration given to a safety factor for hemolytic effects.

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